B=The peak height of the working standard.

The peak height is obtained from the polarogram by measuring the vertical distance from the peak to the baseline of the sample or working standard.

[44 FR 20664, Apr. 6, 1979, as amended at 47 FR 20756, May 14, 1982]

§ 436.325 High pressure liquid chromatography assay for vidarabine.

(a) *Equipment.* A suitable high pressure liquid chromatograph, such as a Waters Associates Model 244[1] or equivalent, equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A 30-centimeter column having an inside diameter of 4 millimeters and packed with a suitable octadecyl bonded silica phase packing such as Waters Associates, Micro-Bondapak C18.[1]

(b) *Mobile phase.* (1) Transfer 2.2 grams of sodium dioctyl sulfosuccinate and 10 milliliters of glacial acetic acid to a 1-liter volumetric flask. Dissolve with 500 milliliters of methanol, dilute to volume with distilled water, and mix. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter.

(2) De-gas the mobile phase just before its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh approximately 24 milligrams of sample or working standard into a 200-milliliter volumetric flask. Add about 150 milliliters of distilled water and heat on a steam bath for 10 minutes. Shake until all the powder is dissolved. Cool to room temperature and dilute to volume with distilled water.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the sample or working standard solution prepared as directed in paragraph (d) of this section into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. The elution order is void volume, 9-β-D-arabinofuranosylhypoxanthine (if present), vidarabine, and adenine (if present).

(f) *Calculations.* Calculate the vidarabine content as follows:

$$\text{Micrograms of vidarabine per milligram} = \frac{A \times W_s \times f}{B \times W_u}$$

where:

A=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);

B= Area of the standard peak;

W_s=Weight of standard in milligrams;

W_u=Weight of sample in milligrams; and

f=Potency of standard in micrograms per milligram.

[44 FR 30334, May 25, 1979, as amended at 47 FR 23708, June 1, 1982]

§ 436.326 Thin layer chromatographic identity test for cefoxitin sodium.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a), (b), (c), (d), and (e) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman #3 MM, chromatographic paper or equivalent.

(2) *Plates.* Use a 20×20 centimeter thin layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers.

(b) *Developing solvent.* Mix ethyl acetate, pyridine, *n*-butanol, acetic acid,

[1] Available from Waters Associates, Inc., Maple St., Milford, MA 10757.